

Pathological investigation of late embryonic death in ostrich hatcheries and their economic impact in Egypt

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Objective

To investigate the pathological causes of late embryonic death in ostrich hatcheries and their economic impact in Egypt.

Methods

The study was conducted from February 2023 through December 2024. The pathological causes, microbiological analysis, and financial impact of late embryonic deaths across 9 ostrich hatcheries in Egypt were investigated.

Results

A total of 1,250 fertile ostrich eggs failed to hatch. The highest mortality rate was observed in Ismailia at 28.88%, whereas the lowest was recorded in Giza at 15.62%. The cumulative economic loss across all hatcheries was estimated at 1,750,000 Egyptian pounds. The bacterial infections were the leading cause of late embryonic death, accounting for 813 cases (65.04%), followed by improper incubation conditions, such as elevated temperatures (22.48%), edema (5.12%), malpositioning (3.6%), improper egg turning (2.56%), and fungal infections (1.2%). The most commonly isolated bacterial were *Enterococcus* spp, *Salmonella* spp, *Proteus* spp, and *Klebsiella* spp. Gross post mortem examination of dead-in-shell embryos revealed consistent lesions, including anasarca, SC and visceral congestion, unabsorbed yolk sacs, and malpositioning. Microscopic evaluation of embryos that died due to bacterial infections revealed severe inflammatory changes in multiple organs.

Conclusions

The infectious and noninfectious factors contribute to late embryonic mortality in ostrich hatcheries, with bacterial contamination being the dominant cause. The embryos that died revealed severe pathological lesions in various organs.

Clinical Relevance

The study highlights the urgent need for improved hatchery hygiene, biosecurity, and strict control of incubation conditions to enhance hatchability and reduce economic losses in the ostrich industry in Egypt.

Keywords: ostrich, embryonic death, pathology, economic losses, hatcheries

The ostrich industry has a growing and diversified contribution to the global agricultural economy, valued for its quality meat, durable leather, and

unique feathers. Ostrich meat contains low cholesterol and fat levels but high protein levels, making it a sought-after option as a healthy substitute for red meat in health-conscious markets.¹ Furthermore, ostrich farming requires significantly less water and feed than conventional livestock, making it a viable option in desert regions and contributing to sustainable agricultural practices worldwide.²

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Egypt's ostrich industry is a new economic sector developed after 1998, comprising small and medium farms and some big companies. The financial gain is enhanced by increasing egg production and optimizing the number of chicks.³ The ostrich industry suffers from high embryonic mortality rates during the artificial incubation of eggs, and inefficient water loss from the total eggs leads to low survival. Understanding the development of embryos is crucial for achieving optimum hatching success.⁴

Ostrich hatcheries are a staple of ostrich farming and are integral to ensuring consistent quality, health, and productivity among ostrich populations. Specialty hatcheries provide strict environment control crucial to successful incubation and hatching, significantly improving hatch rates and reducing chick mortality compared to natural hatching methods.² Hatcheries offer the ideal conditions for embryo development by regulating temperature, humidity, and sanitation. This results in healthier, stronger chicks with better survival and growth potential. Hatcheries are also genetic selection and improvement centers.⁵ Through hatchery breeding programs, farmers can select parents with favorable traits, such as fast growth, high meat percentage, strong immunity, and good leather qualities. This selective breeding helps maintain and improve the overall quality of ostriches through generations.⁶

Hatcheries also have rigorous biosecurity measures in place to ensure that the introduction and dissemination of diseases are avoided, which is essential for safeguarding individual farms and the larger ostrich farming industry. Centralized hatcheries can provide chicks to multiple farms, which helps maintain uniform stock and supports the growth of the ostrich farming industry.⁷ Where ostrich rearing is under development in a particular region, hatcheries play an important role in producing healthy, quality chicks, thus promoting local economic development and farm diversification. Ostrich hatcheries are vital to ensuring the industry's long-term viability, enhancing production efficiency, and responding to the growing global demand for ostrich products.⁸

Late embryonic mortality in ostrich hatcheries is a significant problem that can lead to serious economic losses and reduced hatchability percentages.⁹ The causes of this problem are numerous, but most are a consequence of poor management practices, environmental stresses, and biological deficiencies.¹⁰ Incorrect incubation conditions, specifically temperature and changes in humidity level toward the end of embryonic development, are the most important causes. Ostrich embryos are highly vulnerable, and slight variations from optimal circumstances will impede development or lead to death at hatching.¹¹ Lack of proper ventilation inside the incubator also reduces oxygenation and carbon dioxide buildup, which are toxic to the growing embryo. In addition, poor egg handling and hygiene increase the probability of bacterial or fungal infestation, which can penetrate the eggshell and infect the embryo.¹²

Nutrient deficiency in the breeding stock, especially the lack of critical vitamins (like A, D3, and E)

or trace elements (like selenium), has the potential to result in embryonic weakness with poorly developed organs, thus making them more susceptible to death in later stages.¹³ Genetic factors, like inbreeding or congenital abnormalities, can also predispose embryos to failure. Furthermore, improper egg turning during incubation can lead to malpositioning, interfering with the embryo's ability to pip and hatch successfully.¹⁴ All of these indicate that it is necessary to implement firm control of incubation parameters, adequate breeder nutrition, firm hygiene practice, and genetic management to minimize late embryonic mortality in ostrich hatcheries.¹⁵

Bacterial and fungal infection in ostrich hatcheries is a prime cause of late embryonic mortality, primarily because of sanitation problems, improper handling of eggs, and less than optimal incubation procedures.¹⁶ Contaminants may pass through the micropores of the eggshell or cracks and soiled surfaces when eggs are laid in unclean environments or not disinfected suitably before incubation. The most prevalent bacterial perpetrators are *Escherichia coli*, *Salmonella* spp, and *Pseudomonas* spp,⁴ with fungi such as *Aspergillus fumigatus*, which commonly causes respiratory infections in embryos.¹⁷ The pathogens can colonize the egg's surface and develop through the inner membranes, causing a systemic infection that interrupts the embryo's normal development and kills it later in development.¹⁸ High humidity and improper incubator ventilation also perpetuate the problem by creating a favorable environment for microbial growth.¹²

Contaminated incubators, egg trays, and handling equipment are chronic reservoirs of infection, especially if disinfection routines are not followed rigorously.⁸ An enlarged yolk sac, yolk retention, and abnormal coloration or odor in the egg indicate embryonic death. Most commonly, the embryo appears normal but neither pips nor perishes shortly after, with a terminal infection.⁴ Prevention of this kind of contamination requires excellent hygiene. It involves proper disinfection and cleaning of all hatchery equipment, providing correct incubation, offering clean nesting material, and ensuring breeder birds' optimal health and sanitation. Similarly, eggs should be collected routinely, kept with clean hands, and treated with approved disinfectants to reduce microbial load before incubation.¹⁹

This study aims to investigate the most common causes of late embryonic mortality among Egyptian ostrich hatcheries, identifying the major microbial causatives with pathological assessment. The study also aims to determine the influence of hatchery management practices on embryo mortality. Additionally, the study seeks to offer practical suggestions based on evidence to enhance safety measures and incubation methods, ensuring better hatching rates and preventing financial losses in ostrich farming.

Methods

Ostrich hatcheries and their location

The study was conducted over a period from February 2023 through December 2024. The selected

ostrich hatcheries were located in various regions of Egypt, including farms in the governorates of Giza, Sharkia, Dakahlia, and Ismailia, where ostrich farming was most prevalent due to suitable environmental conditions and growing commercial interest. These hatcheries were equipped with artificial incubation systems and followed standard hatching protocols.

Hatcheries included in the study were chosen based on reported cases of late embryonic mortality (from the 35th day of incubation until the hatching time, the 43rd day) and accessibility for sampling and examination. Five thousand two hundred fertile egg samples were collected and distributed across 9 ostrich hatcheries, including 1,250 eggs that failed to hatch due to late embryonic death. The hatcheries were selected based on reports of late embryonic death during the final stages of incubation. Eggs were examined post mortem to identify pathological lesions and to collect samples for microbiological analysis. Risk factor analysis associated with late embryonic mortality in ostriches includes improper incubation conditions (high temperature), edema, improper egg turning, malpositioning of the embryo, and bacterial contamination.

Bacteriological examination

For the bacteriological examination, samples were aseptically collected from the internal organs (see below) of the unhatched ostrich eggs, which showed signs of late embryonic death. Using sterile instruments, the eggshells were first surface sterilized with 70% ethanol and flamed before being opened in Laminar flow hoods to prevent external contamination.²⁰ Swabs were taken from each embryo's yolk sac, liver, heart, and peritoneal cavity; inoculated into nutrient broth; and incubated at 37 °C for 24 hours for pre-enrichment. Subsequently, the samples were streaked onto selective and differential media, including MacConkey agar (Oxoid), blood agar (Oxoid), and Xylose Lysine Deoxycholate agar (HiMedia) to isolate and differentiate bacterial species, such as *Enterococcus* spp, *E coli*, *Salmonella* spp, *Proteus* spp, and *Klebsiella* spp. Plates were incubated aerobically at 37 °C for 24 to 48 hours. Colonies were examined for morphological characteristics, and suspected isolates were further identified using Gram staining and a series of biochemical tests, such as catalase, oxidase, indole, triple sugar iron, and citrate utilization tests. Some selected samples were kept for more tests and confirmation using analytical profile index (API20E; bioMérieux) identification kits.²¹

Fungal infection

For the fungal test on *Aspergillus* species, samples were carefully collected from the lungs, air sacs, and yolk sacs of ostrich embryos that had died recently and showed signs of fungal infection, such as thick membranes or visible fungal growth. The external surface of each egg was disinfected with 70% ethanol and flamed before opening to minimize contamination. Tissue samples were inoculated onto Sabouraud Dextrose Agar (HiMedia) supplemented

with chloramphenicol to inhibit bacterial growth. The external surface of each egg was disinfected with 70% ethanol and flamed before opening to minimize contamination. Tissue samples were inoculated onto Sabouraud Dextrose Agar and incubated at 25 °C for 5 to 7 days and observed daily for fungal growth. Fungal colonies were identified based on macroscopic characteristics, such as color, texture, and growth pattern, and confirmed by microscopic examination using lactophenol cotton blue staining to observe fungal structures, like conidiophores and conidia typical of *Aspergillus* spp. The genus or species level was identified based on morphological features and comparison with standard mycological references.²²

Histopathological examination

For histopathological examination, tissue samples were collected from late-dead ostrich embryos. Organs, including the liver, lungs, heart, kidneys, and intestines (duodenum), were carefully dissected using sterile instruments. The samples were immediately fixed in 10% neutral buffered formalin for 48 hours to preserve tissue architecture.²³ After fixation, the tissues were washed, dehydrated through a graded series of alcohols, cleared in xylene, and embedded in paraffin wax. Sections 4- to 5- μ m thick were cut using a microtome and mounted on glass slides. The tissue sections were then stained with H&E. The stained slides were examined under a light microscope (DM500; Leica Microsystems).²⁴

Economic losses

The losses were estimated descriptively by multiplying the number of eggs that failed to hatch at the late incubation stage by the cost per egg (1,400 Egyptian pounds [EGP]) for each hatchery. We then summed these values to calculate the total losses.²⁵

The study was approved by the ethical committee at the Faculty of Veterinary Medicine, Aswan University (protocol No. VM/ASWU 03-01-2023).

Results

The examination results of 5,200 fertile ostrich eggs from 9 hatcheries in 4 areas of Egypt (Giza, Dakahlia, Sharkia, and Ismailia) are shown in **Figure 1** and **Supplementary Table S1**, which reveal a significant difference ($P < .05$) among them. Of these, 1,250 eggs experienced late embryonic death, yielding an overall mortality rate of 24.03% during the final incubation period (35th to 43rd day). The highest rate of late embryonic mortality was recorded in hatchery 9 (Ismailia), at 28.88% (260 of 900), followed by hatchery 6 (Sharkia), at 27.89% (106 of 380). The lowest mortality was observed in hatchery 3 (Giza), at 15.62% (75 of 480). Our results indicated that the economic losses from eggs that did not hatch at the late stage varied from 105,000 to 364,000 EGP across different hatcheries, with total losses reaching 1,750,000 EGP for all hatcheries.

Analysis of 1,250 late dead-in-shell ostrich embryos (**Supplementary Table S2**) revealed that bacterial contamination was the most prevalent

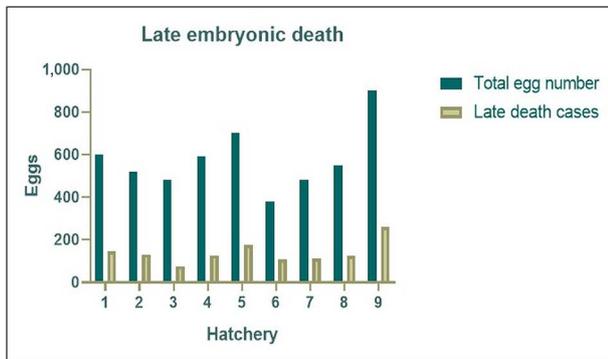


Figure 1—Number of late-death cases compared to total number of fertile eggs.

cause, accounting for 813 cases (65.04%), making it the leading contributor to late embryonic mortality. Improper incubation conditions, particularly elevated temperatures (above 37 °C), were identified in 281 cases (22.48%), suggesting that inadequate environmental control plays a significant role in embryonic demise. Other contributing factors included edema in 64 cases (5.12%), malpositioning of the embryo in 45 cases (3.6%), and improper egg turning in 32 cases (2.56%). Additionally, less frequent fungal contamination was detected in 15 cases (1.2%). Furthermore, the variation between each factor was statistically significant ($P < .05$).

Bacteriological examination

Tests on 813 embryos that died in the hatchery due to confirmed bacterial infections showed that mixed bacteria were common (**Supplementary Table S3**), meaning these infections often involved multiple types of bacteria, with no significant difference ($P > .05$) between the groups. The most common combination was *Enterococcus* spp, *Salmonella* spp, and *Proteus* spp, found in 367 cases (45.14%), indicating a strong link between this group of bacteria and serious infections that led to high embryo death rates. The second most common group was *Enterococcus* spp, *E coli*, and *Klebsiella* spp, seen in 212 cases (26.07%),

showing that *Enterococcus* spp often appears alongside other bacteria. The most common combination of bacteria found was *Enterococcus* spp, *Salmonella* spp, and *Proteus* spp, which appeared in 367 cases (45.14%), indicating that this group of bacteria is strongly linked to serious infections and high rates of embryo death. The second most common group was *Enterococcus* spp, *E coli*, and *Klebsiella* spp, which appeared in 212 cases (26.07%), showing that *Enterococcus* spp often works alongside other organisms. *Escherichia coli* and *Proteus* spp were found together in 119 cases (14.63%), and *E coli* was also identified with *Salmonella* spp in 64 cases (7.87%), often linked to yolk sac infections. The least frequent combination was *Klebsiella* spp and *Proteus* spp, observed in 51 cases (6.27%). These findings add to the complexity of bacterial infections in ostrich embryos, with opportunistic and enteric pathogens playing a major role in embryonic mortality.

Fungal contamination

Fungal analysis of 15 embryos that died during the late hatchery period due to confirmed fungal infections revealed a predominance of *A fumigatus* (100%).

Gross findings

The post mortem examination of 1,250 late dead-in-shell ostrich embryos collected from 9 hatcheries revealed several consistent gross pathological lesions. Many embryos exhibited dehydrated internal organs (**Figure 2**). Generalized edema (anasarca) is characterized by SC fluid accumulation and swollen celom, particularly in the abdomen and pericardial regions. Congestion and multifocal hemorrhages were frequently noted in vital organs, such as the liver, kidneys, and lungs. Unabsorbed yolk sacs and signs of omphalitis (inflammation and infection of the navel area) were common in embryos that died closer to hatching. Malpositioned embryos, particularly those found with the head tucked between the thighs or oriented toward the small end of the egg, were also frequently identified and often associated with poor yolk sac retraction. In some cases, foul odor and discoloration of tissues suggested

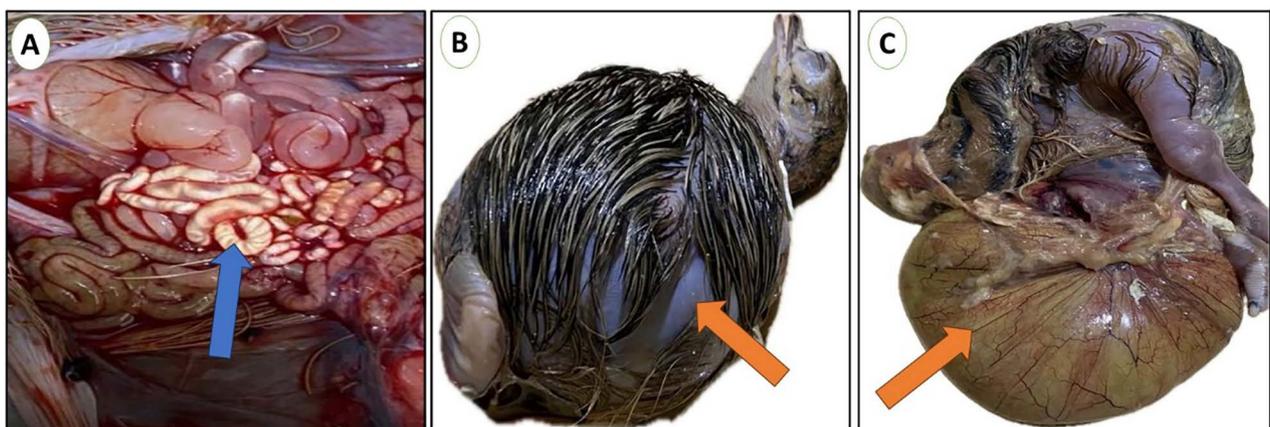


Figure 2—Gross lesions of late-death ostrich embryos. A—Dehydrated intestine (arrow). B—Anasarca (arrow). C—Unabsorbed yolk sac (arrow).

bacterial contamination, particularly when the shell was cracked or pitted. These gross findings indicate a multifactorial pattern of embryonic death.

Microscopic examination

Lung tissues from embryos with a fungal infection caused by *Aspergillus* spp showed typical damage related to aspergillosis. The pulmonary parenchyma exhibited severe congestion, interstitial edema, and dense granulomatous inflammation, often centered around fungal colonies. These granulomas consisted of multinucleated giant cells, epithelioid macrophages, and lymphocytes, forming a wall around the invading hyphae (**Figure 3**). The lung sections, due to bacterial contamination, showed marked congestion of blood vessels, thickening of the interalveolar septa, and prominent infiltration of heterophils and lymphocytes, indicative of acute bronchopneumonia. Alveolar spaces were often partially filled with exudate, suggesting impaired gas exchange in the final days of incubation.

The liver exhibited lesions of severe congestion, hepatocellular degeneration, centrilobular necrosis, and diffuse leukocytic infiltration, primarily composed of neutrophils and mononuclear cells. Small microabscesses were observed in some cases, particularly in embryos infected with *E coli* and *Salmonella* spp (**Figure 4**). The heart displayed myocardial fiber degeneration, interstitial edema, and scattered mononuclear inflammatory cell infiltration, consistent with mild pericarditis and myocarditis.

Examination of the kidneys revealed tubular epithelial necrosis (loss of epithelial nuclei, cytoplasmic hypereosinophilia, and cell sloughing into the lumen), glomerular congestion, and mononuclear infiltration in the interstitial tissue, reflecting systemic infection and potential septicemia (**Figure 5**). The gizzard mucosa was often desquamated, with underlying mucosal necrosis and congestion, especially near glandular tissue. Finally, the intestines (duodenum) showed villous atrophy, goblet cell hyperplasia, and mucosal erosion along with extensive lymphoid

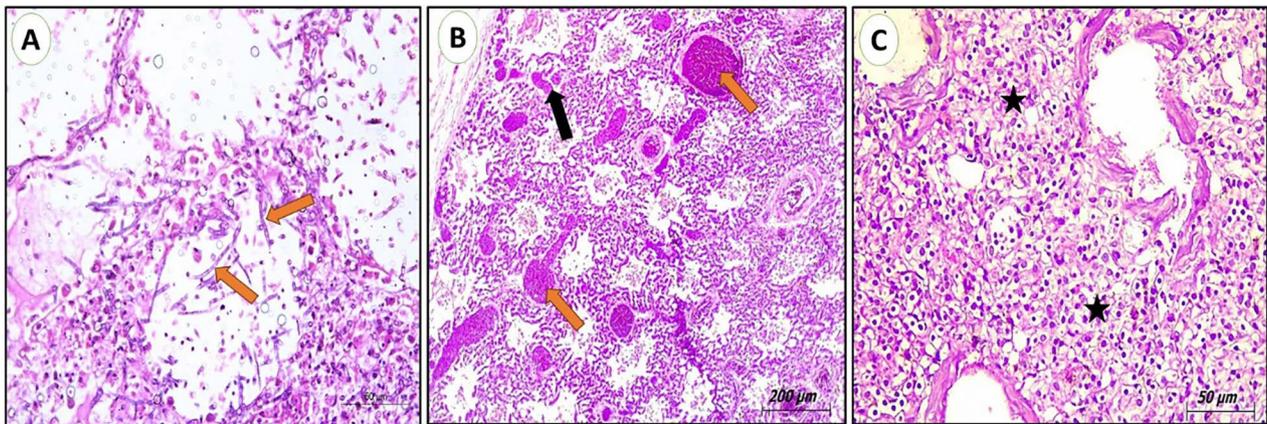


Figure 3—Photomicrograph of lungs from late-death embryos. A—Bronchioles invaded with hyphae (red arrows) with dense infiltration of mononuclear cells (H&E stain; scale bar, 50 μ m). B—Severely congested vasculature (black arrow), and the bronchioles were compacted with blood (orange arrows; H&E stain; scale bar, 200 μ m). C—Intensive infiltration of mononuclear cells (asterisks; H&E stain; scale bar, 50 μ m).

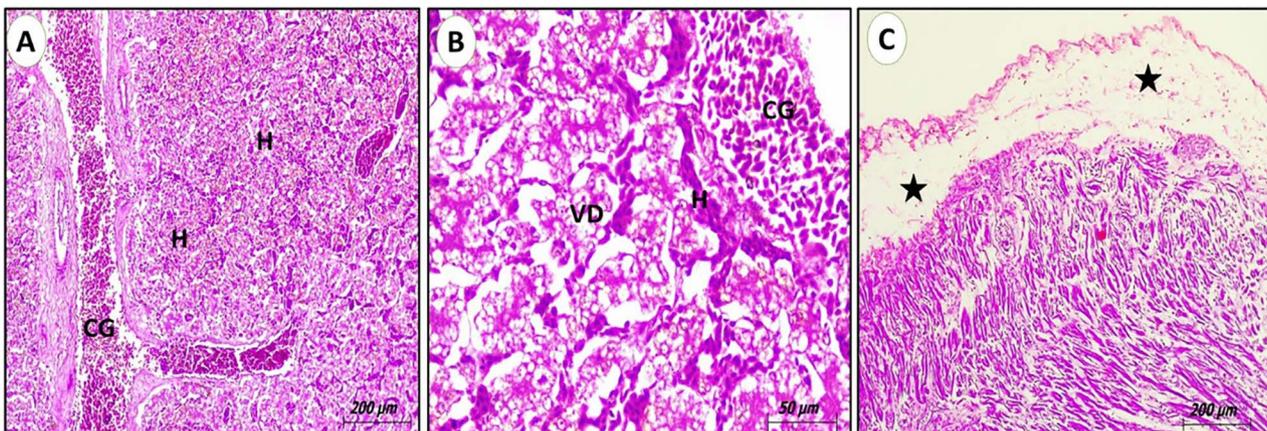


Figure 4—Photomicrograph of liver and heart from late-death embryos. A—The liver showed severely congested central veins and sinusoids (CG; H&E stain; scale bar, 200 μ m). B—Higher magnification of (A) showing hemorrhages (H) as well as vacuolar degeneration (VD) and hepatocellular necrosis (H&E stain; scale bar, 50 μ m). C—Mild hydropericardium (asterisks) and edematous myocardium. CG: Central veins severely congested.

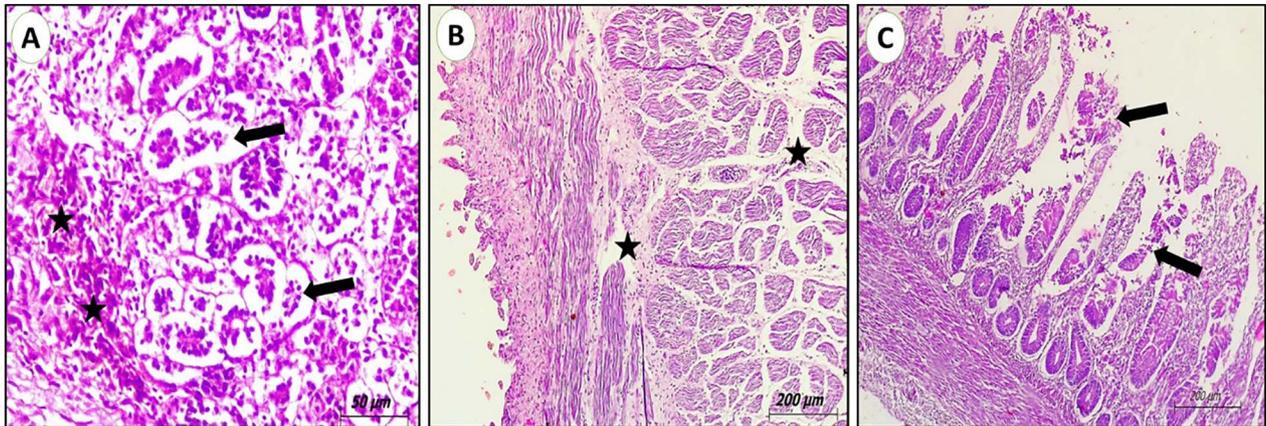


Figure 5—Photomicrograph of the kidney, gizzard, and intestines from late-death embryos. A—Dense interstitial hemorrhages (asterisks) and degeneration of renal tubules (black arrows; H&E stain; scale bar, 50 μ m). B—Submucosal and interstitial edema of the gizzard (asterisks). C—The villi were destroyed and accumulated in the lumen (black arrow; H&E stain; scale bar, 200 μ m).

aggregation in the submucosa, suggesting an active immune response to enteric bacterial invasion.

Discussion

The results of this study highlight a considerable burden of late embryonic mortality in ostrich hatcheries across 4 Egyptian governorates, with a notable impact on hatchery performance and economic viability. Out of 5,200 fertile ostrich eggs examined, 1,250 failed to hatch during the final incubation period (from the 35th to the 43rd day), representing a mortality rate of 24.03%. This death rate is considerably higher than the optimal hatchability targets reported in commercial ostrich production systems.²⁶ The variation in mortality rates between hatcheries suggests disparities in management practices, biosecurity measures, and incubation protocols.¹ Hatchery 9 in Ismailia reported the highest late-stage mortality (28.88%), which may point to more severe or multiple risk factors, such as poor environmental control, high microbial load, or inadequate sanitation. In contrast, hatchery 3 in Giza recorded the lowest rate (15.62%), indicating relatively better handling practices or improved preventive measures.¹

The economic losses associated with this level of embryonic death are substantial, amounting to 1,750,000 EGP across all hatcheries, with individual hatchery losses ranging from 105,000 to 364,000 EGP. These figures underscore the financial vulnerability of ostrich farming to late embryonic mortality and the urgent need to identify and correct underlying causes.²⁷ Given that ostrich eggs have high individual value and longer incubation periods than other poultry species, even small increases in embryo loss can lead to significant economic setbacks. Our results aligned with Abdulkader,³ who concluded that a higher hatching rate leads to greater financial returns and vice versa. Overall, the data show a strong connection between embryo deaths and money loss in ostrich hatcheries, indicating that better cleanliness, safety measures, and control of

incubation could greatly lower embryo losses and improve productivity in ostrich farming in Egypt.

The analysis of 1,250 late dead-in-shell ostrich embryos offers a vital understanding of the multifactorial nature of late embryonic mortality, with a strong predominance of bacterial contamination as the primary cause.²⁸ Accounting for 813 cases (65.04%), bacterial infections emerged as the most significant contributor, underscoring the urgent need to improve hatchery hygiene, egg sanitation, and biosecurity protocols.¹ The common occurrence of infections from multiple bacteria, like *Enterococcus* spp, *Salmonella* spp, *Proteus* spp, *E coli*, and *Klebsiella* spp, shows that there is a serious problem with environmental contamination, poor egg handling, and possibly passing infections from infected parent birds to their eggs. These bacteria are well-known opportunistic pathogens capable of breaching the eggshell barrier, especially when shell integrity is compromised or when storage and incubation conditions favor microbial growth.²⁹ Although fungal contamination was the least frequent finding (15 cases [1.2%]), its presence is still noteworthy, particularly in humid environments where *Aspergillus* spp can thrive.³⁰ The lung granulomatous lesions linked to these infections can greatly affect breathing and are hard to find without a tissue examination, indicating that fungal infections might not be diagnosed as often as they should be.³¹

The bacteriological examination of 813 late dead-in-shell ostrich embryos revealed a high prevalence of mixed infections, underscoring bacterial involvement's complex and multifactorial nature in late embryonic mortality.³² The predominance of mixed bacterial isolates, particularly the combination of *Enterococcus* spp, *Salmonella* spp, and *Proteus* spp, found in 45.14% of cases strongly suggests that synergistic interactions among these pathogens contribute significantly to the severity and lethality of infection.⁴ These bacteria are known for their ability to invade multiple organ systems, cause septicemia, and overwhelm the immature immune defenses of the

developing embryo, particularly during the late stages of incubation when physiological stress is high.³³

The findings of *Enterococcus* spp, *E coli*, and *Klebsiella* spp in 26.07% of cases show that *Enterococcus* spp is a common bacterium found alongside others in ostrich embryo infections. This bacterium, often under-recognized in avian pathology, may facilitate more aggressive gram-negative bacteria by disrupting mucosal barriers or modulating local immune responses. Meanwhile, the identification of *E coli* and *Proteus* spp (14.63%) as well as *E coli* with *Salmonella* spp (7.87%) supports their well-documented role in yolk sac infections, common causes of embryonic and early posthatch mortality in poultry; these results completely agree with Zaki et al.⁴ These pathogens can enter the egg through microscopic shell pores or cracks, especially under suboptimal hygiene conditions in breeder farms or hatcheries.²⁶

Improper incubation conditions, particularly elevated temperatures, were implicated in 281 cases (22.48%) of mortalities, reinforcing the importance of precise environmental control within incubators.³³ Excessive heat during the later stages of development increases metabolic demands, accelerates embryonic growth abnormally, and may compromise the embryo's ability to absorb the yolk sac or assume the correct hatching position.²⁵ Edema, detected in 64 cases (5.12%), is often associated with imbalances in water loss or renal dysfunction and may be secondary to either incubation errors or systemic infections, such as *E coli*.¹⁸ Malpositioning, noted in 45 cases (3.6%), and improper egg turning, noted in 32 cases (2.56%), were linked to mechanical failures or human error in egg handling, leading to poor orientation, insufficient gas exchange, and failure to hatch. These issues are preventable with proper training, automated turning systems, and routine equipment maintenance.³⁴

The gross pathological findings observed during the post mortem examination of 1,250 late dead-in-shell ostrich embryos highlight the multifactorial nature of embryonic mortality. One of the most striking observations was the presence of dehydrated internal organs, a clear indicator of incubation-related stress, most likely due to elevated temperatures or improper humidity control during the final stages of development.¹⁴ This dehydration may impair organ function and viability, especially in metabolically active tissues, such as the liver and kidneys.²⁵

In contrast, many embryos also exhibited generalized edema (anasarca), with notable SC fluid accumulation and swollen body cavities, particularly in the pericardial and abdominal regions. Both dehydration and edema in different cases suggest a disruption in fluid balance regulation, potentially triggered by renal dysfunction, cardiovascular compromise, or systemic infection.³⁵ Moreover, the severe generalized edema in 5.12% of ostrich embryos was perhaps related to hepato-renal and circulatory failure (diffuse necrosis, degeneration, and hemorrhage). Bello et al³⁶ supposed that the main cause of late-stage ostrich embryonic death was circulatory failure associated with generalized SC edema. The high incidence of unabsorbed yolk sacs and omphalitis,

particularly in embryos close to hatching, points to failures in the final stages of yolk absorption, which are critical for providing energy and immunity to the chick. These findings are strongly associated with bacterial contamination, especially by enteric pathogens, such as *E coli*, *Salmonella* spp, and *Proteus* spp, which are known to colonize the yolk sac and cause inflammatory responses.^{4,37}

Publications about ostrich pathology are rare, but pathological studies on embryos in other avian species supported this study's findings. Histopathological lung examination due to fungal infection, specifically caused by *Aspergillus* spp, revealed characteristic lesions of pulmonary aspergillosis. Granulomatous inflammation in the lung parenchyma, centered around fungal colonies, is consistent with chronic fungal invasion.³⁸ The granulomas, composed of multinucleated giant cells, epithelioid macrophages, and lymphocytes, represent a cellular attempt to contain and isolate the invading hyphae.³¹ In contrast, lungs affected by bacterial infections demonstrated features consistent with acute bronchopneumonia, including marked vascular congestion, thickened interalveolar septa, and heavy infiltration of heterophils and lymphocytes. The partial filling of alveolar spaces with inflammatory exudate suggests severe impairment of gas exchange, likely leading to respiratory failure in the final days before hatching.³⁹ These changes reflect acute, rapidly progressing infections, often resulting from high pathogen loads or virulent bacterial strains, such as *E coli* and *Salmonella* spp.⁴⁰

The liver was also impacted, exhibiting hepatocellular degeneration, centrilobular necrosis, and diffuse leukocytic infiltration, indicative of systemic infection or septicemia.⁴¹ The presence of microabscesses, particularly in cases involving *E coli* and *Salmonella* spp, further confirms the invasive nature of these pathogens. Cardiac tissue also showed significant pathological changes, including myocardial fiber degeneration, interstitial edema, and mononuclear cell infiltration, suggesting mild pericarditis.³⁵ While less commonly documented in avian embryos, cardiac involvement in systemic infections reflects the advanced stage of disease and contributes to circulatory failure.

The kidneys displayed clear evidence of systemic impact, with tubular epithelial degeneration, glomerular atrophy, and interstitial infiltration. In the gizzard, notable findings included mucosal desquamation and congestion in the glandular zones, pointing toward localized enteric infections likely ascending from the gastrointestinal tract. Similarly, the intestines showed signs of villous atrophy, goblet cell hyperplasia, and mucosal erosion, with prominent lymphoid hyperplasia, indicating a vigorous immune response to enteric bacterial invasion.⁴²

This investigation demonstrates that late embryonic mortality in ostrich hatcheries in Egypt is a significant issue, both biologically and economically, with an overall mortality rate of 24.03% and an estimated financial loss of 1,750,000 EGP across 9 hatcheries. Bacterial infection was the dominant cause of late

embryonic death, accounting for 65.04% of cases. A smaller but notable number of deaths were attributed to noninfectious causes, such as improper incubation conditions, edema, malpositioning, and poor egg turning, all of which reflect deficiencies in hatchery management. Additionally, although less frequent, fungal infections are caused by *Aspergillus* spp. These findings emphasize the multifactorial nature of late embryonic death in ostrich embryos, where both infectious agents and incubation errors play crucial roles. Effective control strategies must therefore include enhanced hygienic measures, microbiological monitoring, optimized incubation protocols, and breeder flock health management to reduce embryo losses and improve overall hatchery performance.

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Disclosures

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Supplementary Materials

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